

EFFECTS OF DIET QUALITY AND QUEEN NUMBER ON GROWTH IN LEPTOTHORACINE ANT COLONIES (HYMENOPTERA: FORMICIDAE)

JAY D. EVANS¹ AND NAOMI E. PIERCE²

Department of Biology, Princeton University, Princeton, New Jersey 08544-1003

Abstract.—Laboratory experiments manipulating the diet of colonies of the facultatively polygynous ant, *Leptothorax curvispinosus* (Mayr), demonstrated that carbohydrates and protein have synergistic effects on egg numbers and brood production in colonies of this ant. Colonies receiving insect prey and sucrose grew significantly faster than colonies reared on unlimited supplies of either of these food types alone. This study also measured the effect of queen number on colony growth rates. Because the occurrence of multiple queens might affect colony growth only under certain nutritional conditions, polygynous colonies were reared in each of the three diet treatments. Queen number did not affect colony worker production in any of the three diet treatments; thus, individual queens in polygynous colonies produced far fewer workers per queen than did queens in monogynous colonies. There were no interaction effects between queen number and diet on colony growth. Several colonies which lacked morphologically distinct queens produced workers over the course of the experiment. Using artificially established colonies of unmated workers, we found no evidence for parthenogenetic (thelytokous) reproduction in these colonies.

Key Words.—Formicidae, *Leptothorax*, diet, polygyny, brood development.

Production in social insect colonies generally consists of both a vegetative stage, during which new workers are added to colonies, and a sexual stage involving the release of males and females with the potential to mate. The rate of vegetative growth is determined mainly by workers that have foregone their own reproduction to devote energy to the growth of their siblings (Oster and Wilson, 1978). To date, only a few studies have examined the effect of diet on colony growth during this vegetative phase (Brian, 1973; Buschinger and Pfeifer, 1988; Porter, 1989). Buschinger and Pfeifer (1988) found increased larval and pupal production in colonies of *Leptothorax acervorum* fed insect prey and honey, when compared to those fed an artificial diet. They also found behavioral changes in these ants and in the slave-making species *Harpagoxenus sublaevis*. Porter (1989) demonstrated that a diet of insect prey and sucrose was optimal for colony growth in the imported fire ant, *Solenopsis invicta*. Colonies fed insect prey alone fared significantly better than those reared on sucrose alone. Colonies of *Myrmica rubra* reared on insect prey and a sucrose solution also were more productive than those not given insects (Brian, 1973).

The facultatively polygynous ant *L. curvispinosus* is an excellent subject for measuring colony growth and development under controlled circumstances, due to its

¹ Current address: Department of Biology, University of Utah, Salt Lake City, Utah 84112.

² Current address: Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138.

small colony size and the ease with which it accepts artificial nests. Natural populations of these ants are composed of at least three kinds of nests: those having a single queen, those with two or more queens and those containing workers but no queens (Talbot, 1957; Herbers, 1984, 1986 for *L. longispinosus*). Therefore, one can measure the effects of queen number on growth in unmanipulated colonies.

This study had two initial goals. The first was to examine the effect of diet on the population dynamics of monogynous and polygynous colonies of *L. curvispinosus* by rearing them for 14 weeks on three different diets. Colonies were fed either insect prey alone (high protein diet), a sucrose solution (high carbohydrate diet), or both. The second was to observe whether the effect of queen number was the same in colonies reared on the different diets. Eggs, larvae, and workers were censused weekly to determine diet and queen effects on all life stages.

A substantial fraction of nests in the field contained no distinct queen. Eggs and larvae were present in these nests, suggesting either worker reproduction or egg laying by queens in neighboring nests of the same colony. We reared eight colonies which lacked a morphologically distinct queen on the high protein-high carbohydrate diet. Over a period of four months, members of three of these colonies laid eggs that developed into new workers. To determine whether these workers arose via parthenogenesis (thelytoky) from unmated workers, we established and monitored nests of newly eclosed (unmated) workers.

METHODS

Colonies of *L. curvispinosus* were collected from acorns (*Quercus rubra*) in a wooded area on the northeast corner of the Princeton University campus, Princeton, New Jersey. Whole, intact acorns are impenetrable for the ants and they usually rely on acorn weevils (Curculionidae) to make the nuts habitable (Talbot, 1957). These weevils develop within fallen acorns, feeding on the fruit and finally drilling a small exit hole after eclosion. Acorns with holes indicating the emergence of curculionid beetles were taken in dark canisters to the laboratory, where they were examined for ants. Acorns having these characteristic openings usually contained colonies of *L. curvispinosus*.

Ants were placed into individual arenas consisting of clear plastic boxes (11 × 11 × 4 cm) with lids. Fluon (Northern Chemical Co., Woonsocket, R.I.) was applied to the container walls to prevent ants from escaping. Artificial nests were placed into each arena. Nests consisted of two glass microscope slides (2.5 × 7.5 cm) separated by a 1.0 mm wide piece of cardboard cut to form a rectangular cavity between the slides. A single entrance hole was cut along the walls of this cardboard spacer, and a layer of red cellophane was placed between the cardboard and the top slide to reduce light in the nest cavity, while allowing colony observation. Colonies usually moved into the artificial nests within ten hours of being placed into the arena. They were maintained at a temperature of 25°C, on a 16L:8D light cycle.

Following collection, queenright colonies were divided by queen number. Thirty six monogynous colonies were divided into three groups of twelve. Similarly, 15 two-queened colonies were divided into three groups of five. For each queen class, one group received the high protein diet, another received the high carbohydrate diet and a third group was given both. Colonies of similar size were randomized before

Table 1. Egg, larva and worker numbers from initial census, October 30, 1987.

	No queen (N = 12)		One queen (N = 32)		Polygynous (N = 19)	
	\bar{x}	(SE)	\bar{x}	(SE)	\bar{x}	(SE)
Eggs	10.33	(5.08)	21.84	(5.59)	32.68	(5.22)
Larvae	25.75	(5.91)	25.84	(3.57)	38.00	(6.48)
Workers	43.58	(7.66)	46.38	(5.38)	63.95	(10.64)

being placed into the three diet treatments, so that mean colony size was equal across treatments.

Colonies receiving the carbohydrate diet were fed a solution of 10% sucrose. This was dispensed through a capped 1.5 ml. micro-centrifuge tube with a 3–4 cm long (0.25 mm diameter) capillary tube inserted through the side wall. To prevent pressure locks, small holes were made in the caps of the microcentrifuge. Colonies not receiving sucrose were given identical feeders filled with distilled water. All colonies were also provided water via micro-centrifuge tubes capped at one end with cotton. Sucrose solutions and water were replaced throughout the experiment as needed. In the high protein diets, adult fruit flies (*Drosophila melanogaster*) were given to the colonies *ad libitum*. All flies were killed by freezing before feeding them to the ants, as a moving stimulus was not necessary to induce attack by *Leptothorax* workers. Uneaten flies were replaced with fresh flies every two days.

Production of brood and workers within each colony was estimated by taking weekly standing counts. For the censuses, nests were removed from their arenas, and eggs, larvae and workers were counted using a binocular microscope.

We compared the final counts of eggs and larvae using a two-way analysis of covariance (ANCOVA), with diet and queen number as factors and colony size (worker number) as the covariate. Change in worker number was also compared using an ANCOVA, with queen and diet classes as factors and initial worker number as the covariate. In both cases, interactions between queen number and diet effects were also examined. Six of the smaller monogynous colonies went extinct during the experiment and one colony lost its queen in the second week. These seven colonies were excluded from the analysis of worker production.

Colonies of unmated workers were established by periodically harvesting pupae from several productive queenright colonies. When these pupae eclosed, colonies were reared on a diet of *Drosophila* and a 10% sucrose solution. They were maintained at constant temperature (25°C) for five months, then were kept at approximately 4°C for three months, before being returned to room temperature.

RESULTS

Field collections. Table 1 presents the population structure for 63 queenless, monogynous and polygynous colonies. Twelve (19%) of the acorns had no morphologically distinct queen. Thirty-two (51%) had one queen, while nineteen (30%) were polygynous. Three additional colonies collected with this sample consisted of *L. curvispinosus* workers enslaved by queens and workers of the slave-making ant, *Protomognathus americanus*. These colonies were excluded from the experiment.

In the first census, egg presence appeared to be positively correlated with queen

number, although these differences are not significant when confounding effects of colony size are removed (ANCOVA, $P = 0.244$). Eight of the twelve queenless colonies lacked eggs, as did eleven of the thirty-two single-queen colonies. All polygynous colonies had eggs present.

All colonies, including those without queens, contained larvae. These larvae were predominantly early instars and no pre-pupae nor pupae were present. No male or female sexuals were found in any of the colonies censused. From their colony composition, we assume that these colonies were preparing to diapause for the winter. Typically, colonies of *L. curvispinosus* require a period of vernalization before recommencing healthy brood production. However, in the following growth experiments, colonies resumed growth in the laboratory without overwintering. This suggests that, at least in certain parts of its range, *L. curvispinosus* does not have an obligate vernalization requirement.

Standing egg counts. Beginning by the fourth week, colony egg numbers showed the effects of their diets, as shown in Figure 1a. Colonies receiving flies only and those fed sucrose only had similar increases in egg numbers during the experiment, while those receiving both flies and sugar showed a much greater increase. By the end of the experiment, the effects of these three diet treatments were significant (Table 2). Monogynous and polygynous colonies had similar egg numbers throughout the experiment. When analyzed individually, none of the diet treatments showed any effect of multiple queens on egg numbers.

Standing larva counts. As was the case for eggs, larval numbers were highest in the combined fly and sugar treatment. Interestingly, larval numbers were higher in the sugar-only than in the flies-only treatment (ANCOVA, $P < 0.01$). As with the data from egg numbers, queen number did not significantly affect the count totals, and there were no interaction effects between queen number and diet. As shown in Table 2, final larval numbers depended significantly on worker number from the first census.

Production of workers. Worker numbers diverged between the three diet treatments beginning around week 10 (Fig. 1b). These differences occurred six weeks after the egg numbers had begun to show significant differences, giving a rough representation of the development time for workers under these diet and temperature conditions. Production of workers was significantly higher in colonies receiving both flies and sugar than in those receiving flies or sugar alone (Fig. 1b, Table 2). Queen number did not influence worker production in any of the three diet groups (Table 2). Initial colony size was also a poor predictor of colony growth.

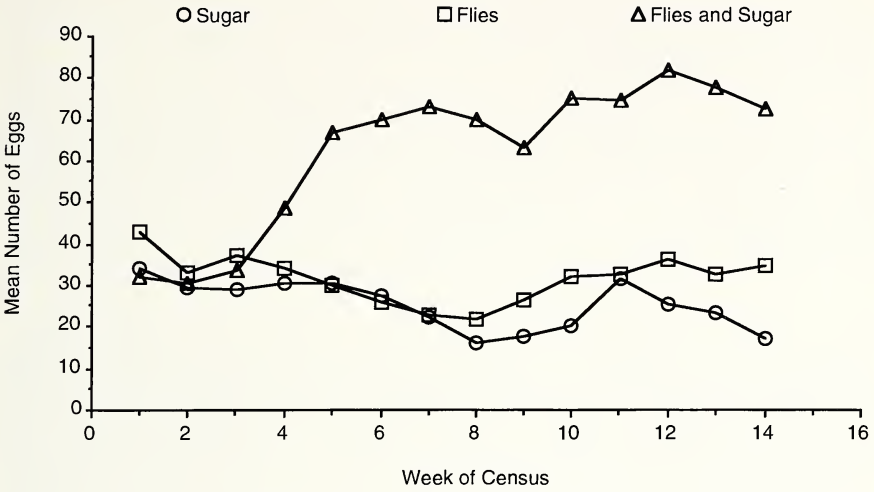
Production in queenless colonies. Of the eight apparently queenless field colonies, six increased in worker number over the course of the experiment (Table 3). In three of these colonies, the increase in worker number was greater than the combined total of eggs and larvae present initially. In 11 artificial colonies consisting of unmated workers, no new workers were produced. Males were produced in each of these colonies, suggesting that laboratory conditions were sufficient for brood development.

DISCUSSION

Diet effects

Diet quality had a strong effect on both egg and larva numbers and overall worker production of these *Leptothorax* colonies. Sucrose (carbohydrate) and insect prey

a)



b)

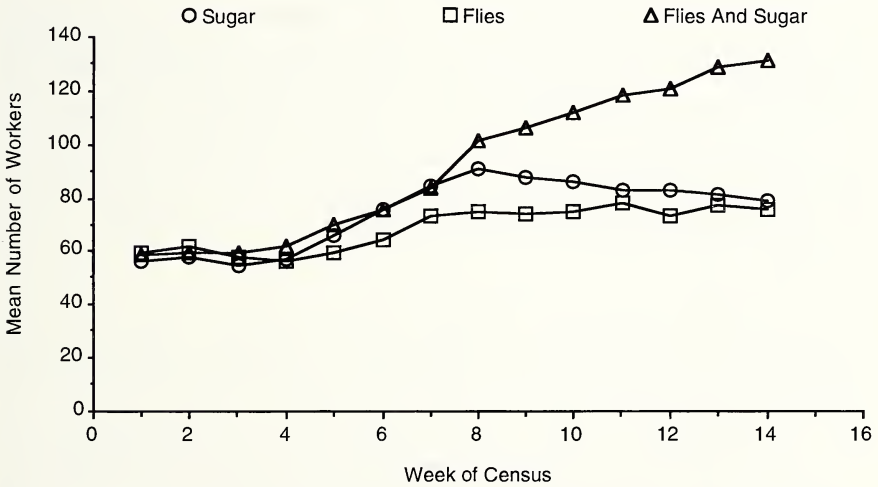


Fig. 1. a) Weekly egg numbers during the experiment for monogynous and polygynous colonies given three different diets. b) Weekly worker numbers during the experiment for monogynous and polygynous colonies given three different diets.

Table 2. ANCOVA results for brood and worker production in experimental colonies. Worker number was used as a covariate against diet and queen number. Egg and larva counts, and worker production, were strongly dependent on diet class. Larva numbers were also positively associated with worker number. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

Source of effect	Nparm	DF	F ratio (Prob > F)		
			Eggs	Larvae	Worker increase
Diet	2	2	6.00 (0.005)**	9.22 (0.0005)***	7.15 (0.0022)**
Queen number	1	1	0.154 (0.697)	0.0046 (0.946)	0.031 (0.860)
Diet × queen number	1	1	0.058 (0.943)	0.554 (0.579)	1.396 (0.244)
Worker number	2	2	0.021 (0.885)	6.58 (0.014)*	0.237 (0.790)

(largely protein) clearly have synergistic effects on egg numbers and subsequent colony growth, as colonies given both grew far more quickly than those raised on an unlimited supply of one or the other. Because a diet of insect prey provides all the nutrients needed for growth, it is surprising that colonies given insect prey alone did as poorly as those fed only sucrose. In contrast, Porter (1989) found that colonies of *Solenopsis invicta* reared on crickets alone grew substantially faster than those given sucrose alone. Other studies (e.g., Buschinger and Pfeifer, 1988; Tschinkel, 1988) also suggest that protein availability is the main determinant of colony growth. It is possible that these colonies of *L. curvispinosus* rely to a larger extent on energy derived from carbohydrates, than do other species.

The data on egg numbers do not provide information about actual egg-laying rates: rather they are a record of the fraction of eggs at each census that has survived oophagy, and it is possible that oophagy varied among the treatments. Brian (1957) found high rates of oophagy by first instar larvae in colonies of *Myrmica rubra*. Assuming that egg hatching time was constant for all treatments, these counts provide an accurate measure of the relative number of potential offspring among the treatments. Accordingly, differences in egg numbers were correlated with the actual worker production of colonies raised on the three diets (corr. coefficient, $r = 0.336$, $P < 0.05$).

Table 3. Census results for eight queenless colonies, taken over 18 weeks. * = Colony produced more workers than the number of initial brood. > = Colony worker numbers increased at some point during experiment.

Colony	11/20/1987				1/2/1988				4/2/1988			
	Eggs	Larvae	Workers	Total	Eggs	Larvae	Workers	Total	Eggs	Larvae	Workers	Total
1*	3	11	48	62	91	31	59	181	97	115	79	291
2*	17	6	31	54	30	41	31	102	—	62	68	130
3*	21	9	23	53	—	104	23	127	33	60	64	157
4>	—	4	4	8	—	4	4	8	—	3	5	8
5>	—	24	37	61	—	20	40	60	—	18	36	54
6>	22	4	21	47	7	15	26	48	1	6	34	41
7	17	9	4	30	2	21	2	25	—	7	1	8
8	28	12	54	94	94	29	51	174	7	29	38	74

Queen effects

The laboratory experiment suggests that queen number does not strongly affect colony growth. If we assume that each queen in a polygynous nest lays roughly the same number of eggs as every other queen in that nest, then the individual fecundity of queens in terms of worker production appears to be substantially reduced by polygyny. These results are consistent with those collected by Wilson (1974) for *L. curvispinosus* colonies monitored over the course of one month. Elmes (1973) found that, while the standing crop of eggs in field colonies of *Myrmica rubra* increased with queen number, the number of larvae, hence colony growth, varied only with the number of workers. He predicted that colony growth in *M. rubra* reflects mainly the amount of food taken in, not the number of queens in a nest.

Further behavioral studies are needed to determine whether the reduction in per-queen productivity is due to inhibition among queens (as shown for *Solenopsis invicta* by Vargo, 1992) or to limits imposed by the size of the worker force. Bourke (1993) found no evidence for such inhibition in *Leptothorax acervorum*. Behavioral and genetic analyses also would help to determine whether the "costs" of polygyny are shared equally within the nest. For example, in polygynous colonies of the imported fire ant, *Solenopsis invicta*, particular queens appear to dominate sexual production (Ross, 1988).

Various colony-level advantages from polygyny are possible. Members of polygynous colonies might benefit from a higher survivorship rate of these colonies as opposed to monogynous colonies. In fact, in the course of this experiment, 7 of the monogynous colonies went extinct, while all of the polygynous colonies survived. While this result may be confounded by differences in worker number, there was overlap in colony size between some of the monogynous colonies that went extinct and the smallest polygynous colonies.

Another explanation for polygyny in ants is an increased ability to exploit newly available resources. For example, polygyny is present in some species of *Pseudomyrmex* inhabiting the hollow thorns of tropical *Acacia* trees. Janzen (1973) suggested that polygynous colonies are more efficient at providing eggs for their exponentially increasing *Acacia* nest sites. Although this has not been examined experimentally, these colonies may use polygyny as a method of displacing other, competing, colonies. Perhaps the similar tendency of *Leptothorax* colonies to bud off into new nests (Stuart, 1985; Herbers, 1986) has selected for the high degree of polygyny in these ants. As polygynous colonies spread to new trees or acorns, conflict among queens may be reduced. However, given that queen number had no effect on colony growth in our experiments for each diet regime, it appears that arguments of polygyny as strictly a means of rapid colony expansion are inadequate.

Polygyny in ants raises a series of important evolutionary and ecological questions. Further studies measuring the costs and benefits of polygyny should have at least two goals. First, production rates must be taken under a variety of dietary and environmental conditions. Second, other factors determining both colony and queen longevity under monogyny and polygyny should be examined. Empirical studies of sperm use and limitation by queens might explain in part the lower instantaneous production rates of queens in polygynous colonies, if monogynous queens are often

sperm limited. In addition, polygyny may be favored by other environmental factors which place a premium on colony survivorship (reviewed by Herbers, 1993).

Production in queenless colonies

The production of workers in queenless colonies remains enigmatic. We were unable to show diploid production in colonies with definitively unmated workers, suggesting that thelytoky is either rare or impossible among these ants. Dissections of workers in naturally queenless colonies, while showing a high frequency of ovarian development (20–40%), did not reveal the presence of spermathecae in the workers examined. While it is possible that queen-worker intermorphs occur in these ants (Passera, 1984), there was no evidence for a stepwise gradation in female size or morphology. This raises the possibility of two distinct castes of mated females; one consisting of winged queens and the other of apterous, mated workers (e.g., Peeters, 1991). Further cytological, genetic or behavior studies are needed to determine the mechanism by which apparently queenless colonies can produce workers in *L. curvispinosus*.

ACKNOWLEDGMENTS

M. F. J. Taylor and L. Keller offered statistical insight. W. D. Hamilton provided advice for the dissections. The comments of A. J. Berry, S. Cover, L. Keller, D. R. Nash, M. Richards and C. Peeters significantly improved the manuscript.

LITERATURE CITED

- Bourke, A. F. G. 1993. Lack of experimental evidence for pheromonal inhibition among queens in the ant *Leptothorax acervorum*. *Anim. Behav.* 45:501–509.
- Brian, M. V. 1957. Serial organization of brood in *Myrmica*. *Ins. Soc.* 4:191–210.
- Brian, M. V. 1973. Feeding and growth in the ant *Myrmica*. *J. Anim. Ecol.* 42:37–53.
- Buschinger, A. and E. Pfeifer. 1988. Effects of nutrition on brood production and slavery in ants (Hymenoptera:Formicidae). *Ins. Soc.* 35:61–69.
- Creighton, W. S. 1950. Ants of North America. *Bull. Mus. Comp. Zool.* 104:1–585.
- Elmes, G. W. 1973. Observations on the densities of queens in natural colonies of *Myrmica rubra* L., (Hymenoptera:Formicidae). *J. Anim. Ecol.* 42:761–771.
- Hamilton, W. D. 1964. The genetical evolution of social behavior I. and II. *J. Theor. Biol.* 7: 1–52.
- Hamilton, W. D. 1972. Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* 3:193–232.
- Herbers, J. M. 1984. Queen-worker conflict and eusocial evolution in a polygynous ant species. *Evolution* 38:631–643.
- Herbers, J. M. 1986. Nest site limitation and facultative polygyny in the ant *Leptothorax curvispinosus*. *Behav. Ecol. Soc.* 19:115–122.
- Herbers, J. M. 1993. Ecological determinants of queen number in ants. *In* L. Keller (ed.), *Queen number and sociality in insects*, 262–293. Oxford Univ. Press, Oxford.
- Hölldobler, B. and E. O. Wilson. 1977. The number of queens: an important trait in ant evolution. *Naturwissenschaften* 64:8–15.
- Janzen, D. H. 1973. Evolution of polygynous obligate acacia-ants in western Mexico. *J. Anim. Ecol.* 42:727–750.
- Keller, L. and L. Passera. 1990. Fecundity of ant queens in relation to their age and mode of colony founding. *Ins. Soc.* 37:116–130.

- Keller, L. and L. Passera. 1992. Mating system, optimal number of matings, and sperm transfer in the Argentine ant *Iridomyrmex humilis*. Behav. Ecol. Soc. 31:359–366.
- Nonacs, P. 1988. Queen number in colonies of social Hymenoptera as a kin-selected adaptation. Evolution 42:566–580.
- Oster, G. F. and E. O. Wilson. 1978. *Caste and ecology in the social insects*. Princeton Univ. Press, Princeton, 352 pp.
- Passera, L. 1984. *L'organisation sociale des fourmis*. Privat, Toulouse, 360 pp.
- Peeters, C. 1991. Ergatoid queens and intercastes in ants: two distinct adult forms which look morphologically intermediate between workers and winged queens. Ins. Soc. 38:1–15.
- Porter, S. D. 1989. Effects of diet on the growth of laboratory fire ant colonies (Hymenoptera: Formicidae). J. Kans. Ent. Soc. 62:288–291.
- Ross, K. G. 1988. Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera:Formicidae). Behav. Ecol. Soc. 23:341–355.
- Talbot, M. 1957. Population studies of the slave-making ant, *Leptothorax duloticus* and its slave, *Leptothorax curvispinosus*. Ecology 38:449–456.
- Tschinkel, W. R. 1988. Social control of egg-laying rate in queens of the fire ant, *Solenopsis invicta*. Physiol. Ent. 13:327–350.
- Vargo, E. L. 1992. Mutual pheromonal inhibition among queens in polygyne colonies of the fire ant *Solenopsis invicta*. Behav. Ecol. Soc. 31:205–210.
- Wilson, E. O. 1974. The population consequences of polygyny in the ant *Leptothorax curvispinosus*. Ann. Ent. Soc. Am. 67:781–786.

Received 23 March 1995; accepted 19 May 1995.